THE OHIO JOURNAL OF SCIENCE

VOL. XXIX MAY, 1929 No. 3

THE ORIGIN OF THE PHARYNGEAL TEETH OF THE CARP.

(CYPRINUS CARPIO LINNÆUS)*.

LINDEN F. EDWARDS,
University of Illinois.

INTRODUCTION.

The doctrine of the specificity of the germ layers postulates, among other things, that the enamel-organs of the teeth of vertebrates be derived from ectodermal epithelium. The presence of teeth in the oral cavity of vertebrates is accounted for by the invagination of the integument during the formation of the stomodæum. This process would result in the carrying inward of the ectoderm and any of its derivatives such as placoid scales—structures which are said to be homologous with the teeth of vertebrates. Since the dermal denticles or placoid scales of elasmobranchs are comparable as to structure and method of development with their teeth, the latter are usually considered nothing more than highly developed spines of the skin, and, since the teeth of elasmobranchs are morphologically similar to those of other vertebrates, it is inferred that all teeth bear a similar relation to the integument. Furthermore, according to the above mentioned doctrine, the epithelial lining of the digestive tract including the pharynx is derived from endoderm. Therefore, the fact that placoid scales are present in the pharyngeal cavity of certain elasmobranchs and the fact that pharyngeal teeth occur in many teleosts raises questions as to the validity of this doctrine and at the same time casts doubt on the origin of such structures.

*Dissertation presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the Ohio State University.

The difficulties of determining the boundaries of ectoderm and endoderm in the oro-pharyngeal cavity of fishes after the formation of the oral and pharyngeal clefts constitutes a considerable obstacle in attempting to solve the problem of the origin of these supposed ectodermal derivatives in this endodermal territory. Nevertheless, extensive investigation has been carried out and various theories have been proposed to account for the presence of these structures in this region. The adherents of the doctrine of the specificity of the germ layers maintain that the presence of teeth in any region is an accurate criterion for the existence of ectoderm in that region at some time in development. They assume, therefore, that these structures are derived from ectoderm which has migrated into this region. They hold, furthermore, that it may be determined how far the ectoderm invaginates into the mouth and pharyngeal cavity of fishes by determining the distribution of placoid scales and teeth in such regions. Two suggestions have been offered as to the probable course of this ectodermal migration: first, that after the rupture of the oral plate the invaginated ectoderm continues to grow posteriorly into the pharynx; second, that the lateral invaginations of the ectoderm, which fuse with similar evaginations of the endoderm to form the pharyngeal clefts, grow inward to the pharyngeal cavity. Thus, according to this idea, the dermal denticles in the pharynx of elasmobranchs and the pharyngeal teeth of teleosts are formed in a manner comparable with that of those in the oral cavity.

O. Hertwig ('74) and Gegenbaur ('98) were among the first to advance the theory of ectodermal migration in order to account for the presence of these structures in the pharynx of fishes. According to these authors ectoderm must grow into the pharynx in the case of fishes if placoid scales and teeth are to be considered as derived from it, since endoderm does not possess the capacity to form teeth.

Steinhard ('03) examined the oral and pharyngeal cavities of numerous species of elasmobranchs with the view to determining the form, arrangement and distribution of placoid scales in these regions. He found that in most of the species examined the whole of the oral and pharyngeal cavities, together with the branchial arches, are almost completely covered with these scales and that they often extend as far back as the commencement of the œsophagus. He mentioned that it is difficult

to account for the presence of such structures over an area like the pharynx which is of supposed endodermal origin. He was of the opinion, however, that, although there is a possibility of the endoderm having acquired a capacity to form dermal denticles, their presence in that region is more likely due to a migration of ectoderm.

Imms ('04) studied the structure of the gill-rakers of the Spoonbill Sturgeon (*Polyodon spatula*) and found that there is a striking similarity between them and the teeth of vertebrates. He offered the suggestion (page 29) "that possibly the gill-rakers of *Polyodon* are morphologically the much modified descendants of exoskeletal structures which have migrated along with the ectoderm on to the branchial arches. The fact that the mucous membrane covering the branchial arches is regarded as being endodermal in origin, offers considerable difficulty to any idea that such structures could have developed there independently and in situ . . . . . It is worthy of note that, with regard to the pharyngeal teeth of many fishes, several writers are inclined to believe that their presence is due to a migration of the ectoderm into the cavity of the pharynx. For this reason, and on account of the difficulty of reconciling them with the presence of anything except ectoderm, I would suggest the possibility that the skeletal tissue of the gill-rakers of *Polyodon* has arisen from portions of the gill-clefts, which have migrated on to the inner or pharyngeal margins of the branchial arches. At all events, if any migration of epiblast has taken place, the latter route seems as feasible as a backward migration from the stomodæum."

Following up the suggestion that the gill-rakers of *Polyodon* may perhaps be regarded as modified dermal denticles, Imms ('05) carried out an investigation with the view to determining the distribution of dermal denticles in the pharyngeal cavity of a considerable number of species of elasmobranchs. His results were practically identical with those of Steinhard ('03). He discovered and emphasized the fact that variations occur in the distribution of the denticles in the oro-pharyngeal cavity of these forms. He described the predominating method of distribution as occurring in those species where the denticles are uniformly distributed over the entire mucous membrane of the mouth, pharynx and branchial arches, often extending backwards as far as the opening of the œsophagus. In some species they are wanting from the roof of the mouth and pharynx

in others they are absent from both the roof and floor of the mouth and pharynx and are restricted to the pharyngeal margins of the branchial arches; while in one species he found that denticles have ceased to be developed in the pharynx except on the hyoid and first branchial arch, but, are retained over a considerable area on both the roof and floor of the oral cavity. In a few species denticles have become lost altogether. Since there exists this variation in the distribution of the denticles in the oro-pharyngeal cavity of elasmobranchs, Imms ('05, pp. 47–49) offered the suggestion that these structures are vestigial organs and that the primitive method of distribution is represented in the ancestral forms of existing elasmobranchs, that is, those species in which they are uniformly distributed throughout the oral and pharyngeal cavities. In order to account for the variations in the distribution of the denticles, which are met with in other species, he proposed that they have been derived from this primitive condition through their becoming restricted to certain areas only. He suggested, furthermore, that, although we know nothing concerning the habits of the ancestral vertebrates or for what particular mode of feeding the structure of their mouth was adapted, "it is possible that the seizure, holding, or perhaps even the crushing of the food may have been effected by the movements of the ventral portions of the arches towards the roof of the oral cavity, after the fashion of the hypopharyngeal teeth in connection with the hinder branchial arches in many Teleosts. If there be any truth in this suggestion, it will not be difficult to appreciate the physiological value of an extensive distribution of denticles over the greater part of the oral and pharyngeal mucous membrane in the primitive Vertebrates. With the evolution of special jaws at a later period, the functional denticles would naturally tend to become restricted to them and constitute ordinary teeth, leaving, however, the residue of the stomodael invasion of dermal denticles to become pharyngeal teeth, or gill-rakers, or to remain as vestigial structures, or to vanish altogether." These suggestions are of interest in that they not only emphasize the idea of an extensive ectodermal migration during ontogenetic development but they also offer an explanation for the phylogenetic origin of pharyngeal teeth.

Following up the theory of ectodermal migration in to the oro-pharyngeal cavity, some investigators propose that this region in fishes belongs morphologically to the integument.

O. Hertwig ('74, page 393) maintained that, inasmuch as placoid scales and teeth are known to arise only from ectoderm, and, since they may be distributed throughout the whole of the oro-pharyngeal cavity even up to the commencement of the œsophagus, therefore, this region does not belong to the gut proper but to the outside integument ("die Mund und Schlundöhle nicht dem Darmtractus sondern noch dem äussern Integumente ängehort)." Jacobshagen ('11, '12) likewise supports this view. In order to support this hypothesis, he offered the following evidence: (1) the pharynx possesses an epithelial lining of the nature of epidermis, (2) it is provided with striated muscle, and (3) the presence of such ectodermal derivatives as placoid scales, teeth and taste buds. He held, that, since no such structures as taste buds and teeth have been demonstrated as occurring in a single place of the vertebrate body which is of proven endodermal origin, therefore, the entire foregut belongs to the ectoderm ('11, p. 568 "dass der gesamte Vorderdarm dem Ektoderm ängehort"). Fahrenholz ('15) studied the distribution of placoid scales in the oro-pharyngeal cavity of various species of elasmobranchs and employed the fact of their presence in this region as evidence to support the above mentioned hypothesis. He held that, since there is a sharp cessation of dermal denticles at the commencement of the œsophagus in most of the species examined, therefore, this region of fishes is ectodermal.

Moroff ('02, '04) was, perhaps, the first investigator to furnish any conclusive evidence for the migration of ectoderm into the pharyngeal cavity of fishes. He claimed that in the development of the gill-slits of fishes ectoderm not only plays the leading role but that it also forms the lining of the gill-slits in adult fishes and even extends into the pharynx. Moreover, he offered the suggestion that this explains why teeth and placoid scales are found on the pharyngeal bars of certain fishes, since, as he pointed out, according to all observations entoderm does not possess the ability to form teeth ('04, p. 205 "das Entoderm nach allen Beobachtungen keine Zähne zu bilden imstande ist").

The idea of ectodermal migration to account for the occurence of pharyngeal teeth in teleosts and placoid scales on the branchial arches of elasmobranchs is also suggested in some text-books. Thus, Tomes ('23, p. 3), in referring to the problem of the origin of pharyngeal teeth, said "the probable

explanation of many of the teeth in these positions is that they are derived from lateral invaginations of the epiblast similar in character to the anterior stomodaeum." He adds, however, that this is not certain and that this explanation cannot apply to all the tooth-like structures found in these and "some other positions, for example, those found in the œsophagus of certain fishes (family Stromatidæ) which are situated in positions quite beyond the limits of epiblastic invaginations." Lankester ('09, p. 58) states that the possession of a dermal exoskeleton is a characteristic feature of the Gnathostomata and that it first appears in the form of small tooth-like structures scattered all over the skin. He goes on to explain, with more certainty than is usually manifest, "since the skin grows inwards at the mouth and gill-slits, denticles may be found also inside the buccal cavity, and on the inner surface of the gill-bars."

Some investigators, on the other hand, not only deny any evidence of a posterior migration of the ectoderm after the rupture of the oral plate or of a lateral migration by way of the pharyngeal clefts but also take the view-point that endoderm possesses the capacity per se to form these so-called ectodermal derivatives. Dohrn ('82, '84) not only denied the ectodermal nature of the foregut of fishes and claimed that the whole domain of the oro-pharyngeal cavity as well as its derivatives are developed from endoderm but also disagreed with the conception of an ectodermal invagination during the formation of the mouth and gill slits. Kerr ('02) also denies the necessity of an ectodermal contribution in the development of the teeth of vertebrates. He claimed ('02, p. 424) that in Lepidosiren paradoxa "the tooth germs begin to appear before there are any traces of a lumen in the buccal cavity." Cook and Neal ('21), after a critical study of the successive stages of embryos of Squalus acanthias, claimed (p. 48) "that the pharyngeal cavity is endodermal in its origin and there is little or no inward migration of the ectoderm into the pharynx." In regard to the capacity of the endoderm to form placoid scales in the pharynx of these fishes, they conclude that "endoderm, therefore, it would appear, may give rise not only to sense organs, but to scales which are usually conceived as ectodermal in origin."

Miss Adams ('24) claims that some potentiality for the formation of teeth exists in the endoderm of Amblystoma. In summarizing the results of her study of the stages in normal development of the mouth and tooth germs, she says (p. 361)

that they "demonstrate the presence of endodermal as well as ectodermal enamel organs in the tooth germs of Amblystoma punctatum." As to the significance of these endodermal enamel organs, she points out that the existence of teeth similar in structure, but with enamel organs derived on the one hand from ectoderm and on the other from endoderm, raises the question of the specificity of the germ layers.

Jenkinson ('06), likewise, implies doubt as to the strict specificity of the germ layers. He points out that "muscles, for example those of the skin-glands in Amphibia, may be derived from the ectoderm, and enamel is said occasionally to arise from mesodermal tissue." According to this author, the facts of descriptive embryology might well be deemed sufficient of themselves to warrant us in relinquishing any hope of retaining the morphological significance which for so long has been attached to the germ layers. He is of the opinion that "the germinal layers are not sets of cells universally identical in origin which necessarily and invariably give rise to certain fixed parts of the adult organization, but merely convenient terms for the primordia of the structures of the adult."

Whereas the idea of ectodermal migration to account for the presence of scales and teeth in the pharyngeal cavity of fishes is suggested in some text-books, the thought is implied in others that these structures could have formed from endoderm in situ. Thus, Balfour ('80, p. 638) states that "although the teeth are to be regarded as primitively epiblastic structures, they are nevertheless found in Teleostei and Ganoidei on the hyoid and branchial arches: and very possibly the teeth on some other parts of the mouth are developed in a true hypoblastic region." Wiedersheim ('86, p. 483) points out that in many cases teeth are met with in parts of the oral cavity where they could have been formed only from endoderm ("wo sie sich nur aus rein entodermalen Boden herausgebildet haben können."). Wortman ('87), in describing the development of the teeth of vertebrates from the invaginated stomodael ectoderm, mentions that in many fishes teeth are found far back in the pharynx and, therefore, beyond the limits of the invagination of the integument. He goes on to add, on the authority of Prof. J. A. Ryder, whose statements he considered highly authoritative due to his extensive knowledge of the embryology of fishes, that these teeth "are truly of hypoblastic derivation. If this be true, the generalization that all teeth are modified dermal spines is certainly incorrect."

It is evident, therefore, that the doctrine of the specificity of the germ layers is occasionally doubted and that the literature dealing with the question of the origin of such structures as the pharyngeal teeth of fishes includes more or less speculation. It is striking how varied are the opinions of investigators and how, oftentimes, their interpretations appear diametrically opposed with regard to this problem. In view of this uncertainty in the interpretation of the origin of pharyngeal teeth of teleosts, Prof. Raymond C. Osburn of the Department of Zoology, Ohio State University, suggested to the writer that he undertake an investigation to attempt to determine from which germ layer the pharyngeal teeth of the carp (Cyprinus carpio Linnaeus) originate. The writer wishes to express his indebtedness to Prof. Osburn who offered many helpful suggestions and kindly criticisms. Thanks are also due Mr. E. L. Wickliff of the Ohio Division of Fish and Game and the employees of the U. S. Bureau of Fisheries at Put-in-Bay, Ohio, through whom the material used for this problem was obtained.

Material and Technique.

Three embryological series of *Cyprinus carpio* were employed in the present investigation. Series 1 was obtained in June 1925 at Buckeye Lake, Ohio by collecting eggs which had been fertilized under natural conditions. The eggs of the carp are pale yellowish in appearance and of such translucency as to be easily overlooked in the water. They are perfect spheres and after being in the water for some time swell to about 2 mm. in diameter. When expressed from the body of the ripe female they are extremely adhesive, and, when fertilized in shallow water are often found adhering to aquatic vegetation. These were collected and were supplied with running water from the lake the mean temperature of which was 69.8° F. The age of the embryos was calculated from the time of hatching, since it was impossible to determine the time of fertilization. Specimens of various stages were preserved every hour from the time of hatching up to the age of 72 hours. Thereafter, they were taken at various intervals of 2, 4 or 8 hours for the next 7 days. The remaining specimens were taken daily until 20 days after hatching. The yolk sac was absorbed by the end of the fourth day after hatching. In order to insure normal growth and development, artificial feeding was carried out with plankton

organisms obtained from the lake by means of a tow net, since larval carp are largely plankton feeders. Series 2 and 3 were obtained in June 1926 and '27, respectively, from the carp hatchery which is conducted by the U. S. Bureau of Fisheries at Port Clinton, Ohio. The eggs, after being fertilized artificially by stripping the ripe males and females, were placed in hatching jars. Thus it was possible to have exact data as to the time of fertilization and also as to the age of the developing embryos. In series 2 specimens were preserved every hour from the time of fertilization up to 80 hours after fertilization. In series 3 they were also taken at hourly intervals from 59 hours after fertilization to 160 hours after fertilization.

The period of incubation depends upon the temperature of the water. In series 2 it was 78 hours after fertilization with the temperature at 69° F. and in series 3 it was 88 hours after fertilization with the temperature at 65° F. It is apparent that stage 88 in series 3 is equivalent to stage 78 in series 2, since both represent the same stage of development in that the larvæ are just hatched. As stated above, it was impossible to determine the time of fertilization in series 1. Inasmuch as the temperature of the water during the development of this series was practically identical with that in series 2, and, since the eggs, which were collected in the same locality, hatched between 3 and 4 days after they were placed in running water it has been assumed that stage 1 in series 1, that is those which have just hatched, is equivalent to stages 78 and 88 in series 2 and 3 respectively. Hence, it is possible to supply any missing stages in any of the series if the other series is complete at that point, and, in this manner, to have an uninterrupted series of embryos from the time the eggs were fertilized until 20 days after hatching.

The eggs were fixed in Bouin's fixing fluid or in corrosive sublimate plus 10% acetic acid—Child's method. The embryos up to the time of hatching were removed from the egg membrane before embedding in order to allow greater penetration of the reagents. To facilitate orientation in the processes of embedding and sectioning the younger stages of embryos were stained in toto with eosin. They were embedded in parafin (M. P. 54° C.) and cut in sagittal, transverse and frontal sections of 10 mu. Various stains were employed, but, Delafield's hematoxylin with eosin as a counterstain proved the most satisfactory for clearness of delineation.

In order to determine the germ layer from which the pharyngeal teeth of the carp originate it was necessary to study the mode of formation of the foregut, since the solution of this problem hinges on the derivation of the pharyngeal mucous membrane. For this purpose the series of carp embryos were examined with the view to ascertaining whether or not there is any evidence of migration of ectoderm into this region during development. In order to determine this it was necessary to work out the method of development of the mouth and gill-slits. After having determined the derivation of the mucous membrane of the pharynx, the method of development of the pharyngeal teeth was studied with the view to determining the germ layer from which the enamel organs take their origin.

Many difficulties were encountered in attempting to solve this problem, most of which involved methods of technique. Orientation of the embryos in the embedding material, in order to obtain sections through the proper plane, proved difficult even though the material was first stained in toto. This difficulty made it necessary to section large numbers of embryos before obtaining the proper plane. A considerable number of fixing and staining reagents were tried before satisfactory ones were found, since many fixing solutions cause the yolk to become hard. This hardening of the yolk hampered sectioning, especially in the early stages, as the yolk has a decided tendency to "shell-out" of the parafin ribbon. Many attempts to dissect away the yolk sac before embedding proved unsuccessful, since the yolk was oftentimes so exceedingly hard and the embryos were so fragile. This difficulty was finally overcome to a large extent by placing the eggs, which had been fixed in Bouin's fluid, for a short time in 35% alcohol plus acetic acid, since this tends to soften the yolk. Child's method of fixation also improved this hardening condition.

It was difficult at first to distinguish the boundaries of ectoderm and endoderm in the oro-pharyngeal cavity after the formation of the oral and pharyngeal clefts. Attempts were made to find a stain which would tend to differentiate these layers. The nearest approach to such a differentiation was obtained by staining heavily with Delafield's hematoxylin and destaining by means of acid-alcohol, in which case the ectoderm remained more heavily stained than the endoderm. Intra-vitam stains, such as methylen blue and neutral red, were tried with the idea in mind of staining the ectoderm before it

invaginated to form the oral and pharyngeal clefts. It was hoped that the ectoderm could thus be traced inward in later stages. However, these attempts proved unsuccessful, since these stains are so readily soluble in alcohol in spite of various attempts at fixation. Golovine's method ('02) of fixing neutral red gave promise of success, but, because of the extreme difficulty of maintaining the proper temperatures attempts to use it met with failure.

Origin and Development of the Foregut.

In order to determine exactly what germ layers contribute to the formation of the mucous membrane of the foregut it was necessary to study its development from the earliest stages of germ-layer differentiation. The first evidence of differentiation of the germ layers of the carp was observed 10 hours after fertilization (Fig. 1). At this time the superficial layer of cells of the blastoderm became differentiated by flattening to form what will later become the "epidermal stratum" or covering layer of the epidermis. Subsequent stages show that this layer gradually becomes reduced to a thin membrane which stains deeply. No evidence could be found of its having taken part in the process of gastrulation to form the inner germ layers.

Gastrulation. This process was observed to occur in the carp in the same manner as that described for the Sea-bass (*Serranus atrarius*) by Wilson ('89, p. 218). By 12 hours after fertilization (Fig. 2) the cells at the edges of the blastoderm had formed a well-marked thickening. This thickening could be recognized round the whole of the periphery of the blastoderm, however, at the posterior middle point, which becomes the tail end of the future embryo, it was more pronounced than elsewhere. One hour later (Fig. 3) a sheet of cells, consisting of two or three cell-layers, terminating anteriorly with a free margin, had grown forward from this point. This point is, of course, the dorsal lip of the blastopore. The ingrowing cellular layer is known as the "primitive hypoblast" from which are later differentiated the endodermal and mesodermal layers. The superficial portion of the blastoderm may now be called the ectoderm. It consists at this stage of two well defined layers, the outermost flattened layer or epidermal stratum and an inner layer of three to five strata of closely packed polygonal cells—the so-called sensory or nervous layer.

By 19 hours after fertilization (Fig. 4) the embryo was distinctly marked out as a median longitudinal thickening of the blastoderm. The anterior pole of the blastoderm has grown forward until it has completely invested the yolk sac. It has already reached the posterior pole of the blastoderm and thus forms the ventral lip of the blastopore. The mass of cells which make up this anterior pole or ventral lip was described by Wilson ('89, p. 226) for the Sea-bass as the "secondary caudal mass" which, according to him, "serves as cellular material for the backward growth of the several organs." The closure of the blastopore has not been completed at this stage as is evidenced by the yolk plug. The future head region of the embyro has already begun to be marked off as a thickening of the ectoderm at the anterior region of the embryonic area. The thin sheet of ectodermal cells, which connects the head region and the secondary caudal mass, is the non-embryonic area of the blastoderm. It forms an investment for the yolk sac and is continuous with the ectoderm which covers the embryo. The primitive hypoblast has extended forward until its anterior free margin has come into contact with the ectoderm in front of and ventral to the future fore-brain. It is everywhere clearly marked off from the overlying ectoderm, with the exception of its posterior extremity where it bends round into and blends with the ectoderm at the dorsal lip of the blastopore. For some distance anterior to the blastopore it consists of three to four strata of more or less polygonal cells. As it proceeds anteriorly it gradually becomes reduced to two strata and in the region of the future fore-brain it is only indistinctly divisible into two layers. Flattened cells appear here and there ventral to the primitive hypoblast and in close contact with the yolk. These flattened cells represent the first evidence of endoderm.

The process of endodermal differentiation can best be made out in figure 5, which is a transverse section through an embryo 21 hours after fertilization. The primitive hypoblast has divided on each side of the middle line into two plates of cells, an upper consisting of two to three strata of polygonal cells and a lower unicellular layer of flattened cells. The upper layer of cells represents the mesoderm, which is seen to be made up of separate halves, one on each side of the middle line. The lower flattened layer is the endoderm, which lies in close contact with the yolk. The cells in this layer are observed to

approach a columnar shape in later stages. The median tract of cells has already separated as the primordium of the notochord.

The process of endodermal-mesodermal differentiation was observed to begin in the posterior region of the embryo and to proceed gradually forward. By 23 hours after fertilization (Fig. 6) the endoderm is completely established as a connected unicellular layer, the anterior extremity of which extends up to and comes into contact with the ectoderm near the angle formed by the lower forepart of the head and the anterior ectodermal wall of the yolk sac. The entodermal cells have maintained their flattened appearance in the future brain region. However, they have already begun to assume a columnar shape in the pharyngeal region, which can readily be identified by means of the otic vesicle. The endodermal cells can easily be distinguished from the mesodermal cells, which are polygonal in shape except in certain portions of the head region where they appear as scattered mesoblast cells. The primordium of the foregut is thus established rather early in development. It is evident that up to this time it is unquestionably endodermal in origin, since no openings have been established and consequently no ectodermal migration could have occurred.

A study of the subsequent history of the foregut of the carp reveals some features which are peculiar in a number of respects: (1) the original unicellular layer of endoderm is transformed into a solid cord, which is at first considerably depressed, by a process of folding; (2) a lumen is gradually established by a separation or retreat of the endodermal cells; (3) the oral end of the foregut appears to be developed from behind forwards without any clear evidence of a stomodaeum. In figure 7, which is a transverse section through the pharyngeal region of an embryo 30 hours after fertilization, the endodermal layer is recognizable by means of the columnar shape of its cells. It passes ventral to the primordia of the brain and notochord, on either side of which it rises to form a fold which is directed laterally and dorsally. These obliquely directed folds represent the pharyngeal folds which later contribute to the formation of the gill-slits. From a study of successive stages the base of each fold is seen to grow toward the median line where it fuses with its fellow of the opposite side thus closing in the foregut ventrally. The foregut then consists of

two rows of cells, a dorsal and a ventral, which are at first pressed against each other without any evidence of a lumen between them. This depressed condition of the foregut is no doubt the result of the pressure exerted by the excessive growth of the overlying brain which tends to mold it upon the yolk sac.

Later scattered lumina appear here and there between the two rows of cells. These unite eventually thus establishing the lumen of the foregut. With the appearance of this lumen the endoderm was observed to assume the same structure as the ectoderm; that is, it appeared to be composed of two layers of cells, an inner layer composed of flattened, pavement-like cells and an outer layer of regularly arranged columnar cells. This observation agrees with that of Moroff ('02, p. 336) for Trutta fario. The appearance of this inner flattened layer in the lumen of the foregut raises the question as to its origin. Is it derived from the outer columnar layer by a process of division or delamination or does it represent ectoderm which possibly migrates into this region? In order to answer these questions it was necessary to study the method of formation of the mouth and gill-slits.

A. FORMATION OF THE GILL-SLITS.

Investigators are not in accord as to the exact method of the formation of the gill-slits in fishes. It is generally agreed, however, that the primordia of these clefts arise by paired, obliquely directed folds of the pharyngeal endoderm coming into contact with lateral invaginations of the ectoderm, and, that openings are established by the rupture of the closing-plates which are formed at the point of fushion of the ectoderm and endoderm. The diversity of opinion is in regard to the germ layer which plays the leading role in the process and to the layer or layers which form the lining of the gill-slits in adult fishes. Dohrn ('82, '84) claimed that in Belone embryos the ectodermal invaginations and the endodermal evaginations are simultaneous, but, that the latter are more pronounced, so that the greater part of the lining of the gill-slits is derived from endoderm. Cook and Neal ('21, p. 49) and Balfour ('78, p. 49) agree that in elasmobranchs the endoderm is the active layer in the formation of the gill-slits and that it serves as their lining. Balfour described the method of formation of the gill-slits in these forms as an outgrowth of the pharyngeal

endoderm which "meets the passive external skin, coalesces with it, and then, by the dissolution of the wall separating the lumen of the throat from the exterior, a free communication from the throat outwards is effected . . . . . Thus it happens that walls lining the clefts are entirely formed of hypoblast." He hastened to add that "it should be remembered, however, that after the gill-slits become open, the point where the hypoblast joins the epiblast ceases to be determinable, so that some doubt hangs over the above statement."

Greil ('06), who made a comparative study of the method of development of the gill-slits in Elasmobranchs, Ganoids, Dipnoi and Teleostei, maintained that the ectoderm plays a passive role in the process of gill formation, that it becomes indistinguishable where it blends with the endodermal evaginations, but, that the epidermal stratum grows inward and forms the inner lining of the gill-slits (p. 268 "die Kiemenspalten an ihren Oberflächen von einer ektodermalen Zellschichte überkleidet sind."). According to Moroff ('02, '04) the ectoderm plays the leading role in the development of the gill-slits of Trutta fario. On the other hand, he agrees with Greil that the ectoderm migrates inward to furnish the lining of the gill-slits. He claims, moreover, that the ectoderm continues to grow inward to the pharyngeal cavity. As pointed out in the Introduction, this was his explanation for the origin of placoid scales and teeth which sometimes occur in this supposedly endodermal territory. Oellacher ('73, p. 79), Göette ('01, p. 566), Lankester ('09, p. 58) and Kingsley ('26, p. 270), likewise, support the view that the gill-slits of fishes are lined with an invagination of the integument. Kingsley concludes his description with the remark that "the matter is one of great difficulty, and cannot be regarded as settled." With this thought in mind, and in spite of the fact that the weight of the evidence favors the idea of ectodermal migration by way of the gill-slits, it was deemed necessary to study the method of development of the gill-slits in the carp in order to determine exactly whether or not ectoderm does migrate inward to the pharyngeal cavity by this route.

It was ascertained from a study of transverse sections through the pharyngeal region of the series of carp embryos that six pairs of pharyngeal clefts develop in a consecutive order from anterior to posterior. The most anterior pair, the hyomandibular, which intervenes between the hyoid and mand-

ibular arches, closes soon after its formation and is thus obliterated. Behind this there are five pairs of clefts which form a similar number of gill-slits. Inasmuch as the method of development of the five pairs of pharyngeal clefts is essentially comparable a description of the hyo-branchial or first pair, which intervenes between the hyoid and first branchial arches, should suffice as a means of determining what part the ectoderm plays in their formation.

The gill-slits were observed to arise according to the manner generally described by the majority of investigators for the various forms of fishes, that is, by lateral folds of the pharyngeal endoderm fusing with similar invaginations of ectoderm. The first evidence of the pharyngeal fold, which will assist in the formation of the first gill-slit, was found 30 hours after fertilization (Fig. 7). The position of this fold, ventral and slightly posterior to the otic vesicle, is relatively constant throughout development. The further course of its development will be evident if figure 7 be compared with figures 8 to 15. Whereas the fold had just begun to form 30 hours after fertilization, it was well marked out 2 hours later (Fig. 8). At this stage the apex of the fold has extended laterally and dorsally until it has come into contact with the inner cells of the ectoderm. The latter is recognizable by means of its superficial layer of flat cells, the epidermal stratum, and its inner layer of cuboidal cells, the so-called nervous layer. On the other hand, the endodermal cells, which make up the pharyngeal fold, are columnar in shape, and, are regularly arranged in rows. The two rows of endodermal cells are firmly applied against each other without any trace of a lumen between them. There is no evidence as yet of a simultaneous invagination of the ectoderm, although its inner layer has thickened somewhat at the point where it comes into contact with the apex of the pharyngeal fold. Thus far, at least, the endoderm has, apparently, taken the leading role.

By 35 hours after fertilization (Fig. 9) the inner layer of the ectoderm has thickened considerably at the point of contact with the pharyngeal fold. The latter is still recognizable by means of the regular arrangement of its columnar cells in two rows. The inner ectodermal cells are irregularly arranged and are seen to cover the apex of the pharyngeal fold in a cap-like fashion. Apparently, the outer layer of ectodermal cells has begun to invaginate as is evidenced on one side by a slight

depression at the external surface. One hour later (Fig. 10) the inner layer of the ectoderm was observed to penetrate the apex of the pharyngeal fold as is evidenced by the wedge-shaped plug of cells which can be seen entering the endodermal fold at this point.

In figure 11, which is a transverse section through the pharyngeal region of an embryo carp 39 hours after fertilization, the apex of the pharyngeal fold has become continuous with the inner layer of the ectoderm so that it is no longer possible to determine the boundary between endoderm and ectoderm at this point. Furthermore, a slight cleft-like lumen has appeared in the outer portion of the pharyngeal fold into which the epidermal stratum dips slightly thus forming a shallow funnel-shaped depression at the surface. This potential opening represents the primordium of the first gill-slit.

An examination of subsequent stages reveals that the epidermal stratum continues to grow inward as rapidly as the cleft-like lumen proceeds inward to the pharyngeal cavity. This is evidenced in figure 12, which is a tranverse section through the region of the first gill-slit of an embryo 56 hours after fertilization, by flattened epidermal cells lining the lumen. These are seen to stop abruptly at the point where they come into contact with the depressed foregut which is still in the solid stage. The connection of these flattened cells with the epidermal stratum is readily recognizable at the surface. They are easily distinguished from the original endodermal cells, since the latter are columnar in shape and are regularly arranged in rows. Figures 13, 14, 17 and 18 also present evidence of the migration of the epidermal stratum inward to the pharyngeal cavity. Figure 17 is a photograph of a sagittal section through the pharyngeal region 57 hours after fertilization in which is shown the character of the cells lining the first gill-slit. The section was cut far laterally and consequently the connection of the gill-slit with the foregut cannot be made out. The cell outlines are more or less indistinct, however, flattened cells, which are stained heavily, can be recognized lining the lumen of the gill-slit. These cells are continuous with those on the external surface. The latter appear pavement-like with a large central nucleus as can be seen above and to the right of the external opening of the gill-slit. Figure 13 shows the entire anterior portion of the same embryo as in figure 17 with only the region of the first gill-slit drawn in detail. This was done

under oil immersion for the purpose of demonstrating the relation of the flattened cells, which line the lumen of the gill-slit, to the underlying columnar cells of the endoderm as well as to establish their connection with the epidermal stratum. Figure 14 is a transverse section through the region of the first gill-slit 78 hours after fertilization. The fore-gut as well as the first gill-slit has developed a rather spacious lumen. Not only has the lumen of the foregut become continuous with that of the gill-slit but also the flattened cells, which line the lumen of the latter, pass inward to form the lining of the pharyngeal cavity. These inner flattened cells are identical in appearance with those on the external surface with which they connect. They are easily distinguished from the underlying endodermal cells which are columnar in shape and uniformly distributed. In figure 18, which is a photograph of a sagittal section through the anterior portion of an embryo 80 hours after fertilization, all of the pharyngeal clefts have formed. They appear very much crowded together antero-posteriorly with only narrow cleft-like lumina with the exception of the first or hyo-branchial cleft. Here, as in figure 14, a pronounced lumen has formed and its connection with the pharyngeal cavity is well shown. The flattened cells, which form the lining of this cleft, are seen to pass inward to the pharyngeal cavity where they spread out in all directions. These cells appear heavily stained and can be traced from the pharyngeal cavity outward to the external surface.

It is evident from these observations that the pharyngeal clefts in the carp are formed by pharyngeal folds extending dorsally and laterally until their apices come into contact with the inner cells of the ectoderm which respond by sending inward a wedge-shaped plug of cells. The latter ruptures the apex of the pharyngeal fold which then becomes continuous with the inner cells of the ectoderm. A potential opening is established at the external surface by the growing inward of the epidermal stratum. The latter thus furnishes the inner lining of the gill-slit, and, as it continues to migrate inward forms also the lining of the pharyngeal cavity. The lining of the lumen of the pharyngeal cleft as well as that of the pharyngeal cavity is, therefore, made up of two kinds of cells, an inner flattened layer and an outer columnar layer. Stages 30 to 78 were re-examined with the purpose of determining whether or not these flattened cells could possibly have been derived from

the underlying columnar cells by the process of division or delamination. No such evidence could be found.

As already indicated, the five pairs of pharyngeal clefts do not form simultaneously but develop in sequence from anterior to posterior, the most anterior of which is the first to arise. The remaining pairs of pharyngeal folds were examined with the view to ascertaining whether or not the characteristic flattened cells make their appearance in their lumina before the primordia of the clefts had been established. The course of development was traced as in the case of the hyo-branchial clefts from the time that the folds made their appearance up to the establishment of the primordial clefts. No evidence could be found in any of these where flattened cells appear in their lumina previous to the time when the ectodermal plug of cells penetrates the apices of the folds. Since these flattened cells are of the same character as those in the epidermal stratum, and, since the latter has been shown to extend gradually inward by way of the pharyngeal clefts to the lumen of the pharynx they are undoubtedly ectodermal in derivation. It is negligible, therefore, whether or not ectoderm migrates into the oro-pharyngeal cavity of the carp by way of the mouth, since the inner lining of the pharynx has been demonstrated to be derived from the epidermal stratum which has migrated inward by way of the gill-slits. Nevertheless the method of development will be briefly outlined, since there are some features of considerable interest in connection with its development.

B. FORMATION OF THE MOUTH.

In the higher vertebrates the endoderm is said to form a simple tube, the anterior extremity of which terminates blindly near the anterior end of the body on the ventral side. It is generally conceived that the ectoderm invaginates to form an oral pit or stomodaeum the posterior boundary of which comes into contact with the blind anterior extremity of the foregut thus forming an oral plate. Eventually this ectodermal-endodermal plate ruptures thus establishing the mouth.

According to some investigators the mouth in teleost fishes does not arise in this supposed typical fashion but develops in a manner peculiar to this group. Dohrn claimed, supporting his statements by figures, (Dohrn '82, Plates XV and XVI) that the mouth in Belone, Labrus merula, Gobius, etc. arises in a manner comparable to that of the gill-slits. In his description

of the development of the mouth in these forms he points out the following features: (1) the anterior extremity of the depressed foregut sends out a pair of obliquely directed folds which extend laterally and come into contact with the ectoderm as in the case of the pharyngeal folds situated more posteriorly; (2) clefts form here in the same manner as in the pharyngeal region so that the mouth of teleosts therefore, opens laterally; (3) in the meantime, the anterior extremity of the foregut continues to grow forward until it comes into contact with the inner cells of the ectoderm in the midline ventral to the primordium of the fore-brain; (4) the ectoderm which covers the anterior extremity of the foregut breaks through without any evidence of a stomodaeum. Ryder ('84, p. 529), likewise, supported this view. In reference to Dohrn's contention that the mouth in teleosts is formed by outgrowths, which grow laterally, he stated, "I have seen evidence in a series of embryo Clupeoids which have inclined me to think Dohrn's view the correct one." He was likewise unable to find any trace of a stomodaeum in these forms.

Apparently it is generally assumed that the mouth in teleost fishes develops according to the typical vertebrate method. Most investigators have been concerned with the larval and post-larval development, and, a few have studied the histological changes in the epithelial lining accompanying this development. Stewart ('26) implies, at least, that the mouth in Castostomus commersonii develops in a manner similar to that of higher vertebrates. He described the mouth in the newly hatched larva as being in the oral pit stage and that it does not open until some time after hatching. He mentioned that after the mouth opens the oral and pharyngeal cavities "are lined with a stratified epithelium, the cells of which are rounded, some of the superficial cells being flattened." Wilson ('89) dismissed the problem of the formation of the mouth in Serranus by merely stating that "the mouth breaks through a couple of days after hatching." He, likewise, pointed out that "shortly after the mouth appears, the cells which line the alimentary canal lose their embryonic appearance and come to look much like an adult mucous membrane."

In the carp the mouth breaks through in the angle formed by the lower fore part of the head and the anterior ectodermal wall of the yolk sac. By 79 hours after fertilization (Fig. 19) the foregut has acquired a spacious lumen. From a study of

preceding stages it was ascertained that this lumen develops from posterior to anterior and that it is formed by a retreat or separation of the dorsal and ventral rows of columnar cells which make up the depressed foregut. The mouth has not opened at this stage as is evidenced by the plate of cells which forms the anterior boundary of the foregut. One hour later, however, this oral plate (?) ruptures thus forming the mouth. As can be seen in figure 19 there is no evidence of a stomodaeum at the point where the mouth breaks through. It is true that the angle between the head and the yolk sac—the point where the mouth breaks through—has deepened considerably during development, however, this deepening is not due to an invagination of ectoderm, since there is little evidence of cellular activity, but rather to the forward growth of the head. It is evident from these observations that the statements of Dohrn and Ryder concerning the lack of a stomodaeum in certain teleosts also hold true for the carp. Moreover, as Dohrn maintained, the opening of the mouth occurs from within to the outside.

With the establishment of the mouth the ectoderm becomes continuous at the lips with the inner lining of the oral cavity. It may be assumed, however, that the inner lining of the oropharyngeal cavity is of ectodermal origin before the mouth breaks through, since it has been demonstrated that the epidermal stratum grows inward by way of the pharyngeal clefts to the pharyngeal cavity where it forms its inner lining. These flattened epidermal cells and their relation to the underlying columnar cells can readily be made out in the lumen of the foregut posterior to the oral plate.

An examination of the series of carp embryos also furnishes some evidence to support the views of Dohrn and Ryder as regards the mouth resembling a pair of fused gill-slits in its origin. In figure 15, which is a transverse section cut near the posterior boundary of the optic vesicles 27 hours after fertilization, the endodermal layer, recognizable by the columnar shape of its cells, rises up on either side of the primordium of the fore-brain to form a lateral fold. The apex of the fold on one side has already come into contact with a proliferation of the nervous layer of the ectoderm. The subsequent history of these folds parallels that of the pharyngeal folds as is evidenced in figures 16, 20 and 21. Figure 16 is a sagittal section cut far laterally through the anterior region of an embryo 59

hours after fertilization. Slightly posterior and ventral to the eye is a two-layered fold of columnar cells, the so-called oral fold, which extends obliquely downward and forward to the ectoderm in the angle between the head and the yolk sac. The boundaries of the columnar cells, which are presumably endodermal, and the inner ectodermal cells are no longer distinguishable. A potential opening appears to have been established in the angle between the head and the yolk sac as is evidenced by a slight indentation of the epidermal stratum at this point. In figure 20, which is a photograph of a sagittal section cut far laterally from the same specimen as in figure 19, the epidermal stratum is migrating inward by way of the oral fold, as is evidenced by the thin, heavily stained layer which is interposed between the two rows of columnar cells which make up the fold. Figure 21 is a photograph of a transverse section cut far anteriorly through the same embryo as in figure 14. A fold of columnar cells is seen lying ventral to the eyes and extending laterally to the inner layer of the ectoderm with which it is continuous. The oral end of the foregut is somewhat depressed, however, a slight lumen can be made out which is lined with flattened cells. Although their continuity with the epidermal stratum is not clearly visible in this figure, the same could be made out when examined under oil immersion.

It appears evident from these observations that the mouth of the carp develops in a peculiar manner as compared with that of higher vertebrates. It is questionable whether or not it represents a pair of fused gill-slits or that it merely resembles a pair of gill-slits in its origin. Wilder ('23, p. 296), in discussing the origin of the vertebrate mouth, suggested "that in some form midway between the lamprey eel and the shark the habit arose of seizing and taking in food by the anterior gill-slits, the edges of which, provided with sharp, pointed scales, served better for the retention of their living prey than did the oral hood and horny teeth of the actual mouth." He suggested, moreover, that the continuance of this habit eventually gave rise to the vertebrate mouth, formed by the ventral fusion of the two lateral gill-slits, and to the jaws, formed by the movable gill-arches which were armed with placoid scales. This hypothesis has not received much attention, chiefly, because, it does not show that way in the lowest fishes. Since teleosts are a long way from being primitive, the question

arises does the mouth of teleosts represent a recapitulation or a specialization?

The significance attached to the results of these observations on the development of the mouth of the carp, aside from the evidence that it opens from within outwards, apparently lacking a stomodaeal invagination, and that it appears to resemble a pair of fused gill-slits in its origin, is that it serves as an avenue for entrance of the ectoderm into the oro-pharyngeal cavity. Whether or not these ectodermal cells migrate posteriorly into the pharynx is impossible to determine, since, as already indicated, ectodermal cells migrate inward to the pharyngeal cavity by way of the gill-slits before the mouth is established.

It was ascertained from an examination of later stages that the two types of cells, which form the lining of the oro-pharyngeal cavity, maintain their integrity throughout the further course of development of the foregut. These results are supported by Pictet ('06), who investigated the histological structure of the alimentary tract of the adult carp. He described the mucous membrane lining the oro-pharyngeal cavity as being made up of two distinct layers of cells, an inner squamous and an outer or deeper layer of columnar cells. He found that in the remaining portions of the tract the mucous membrane is made up entirely of columnar cells which resemble those in the lowermost layer of the pharynx.

The evidence furnished by a study of the histological structure of the foregut as well as the embryological considerations presented in this paper are sufficient grounds to prove that the mucous membrane lining this region is composed of two types of cells each derived from a distinct source, an inner layer of flattened cells the presence of which is accounted for by migration of ectoderm, and, an outer layer of columnar cells which represents the original endoderm.

Having established the germ-layer origin of the pharyngeal mucous membrane, the next procedure is to study the development of the pharyngeal teeth with the view to determining which of these two layers of cells give rise to the enamel organs of the teeth.

Origin and Development of the Pharyngeal Teeth.

In the carp, as in all cyprinoids, the jaws are toothless. Teeth occur, however, on the inferior pharyngeal bones (ceratobranchials) of each of the fifth gill-arches for which reason they receive the name pharyngeal teeth. Figure 22 is a photograph of the fifth gill-arches of an adult carp showing the number, form and arrangement of these teeth. Each pharyngeal bone bears 5 teeth placed in three rows and arranged according to a 1, 1, 3 formula. Each tooth consists of a root, neck and crown and is firmly anchylosed to the bone. The masticatory surface is more or less oval in shape with 3 or 4 parallel and slightly serrated furrows.

According to all accounts of the development of the teeth of vertebrates, a tooth always forms in a so-called tooth-germ which consists of two portions, an enamel-organ and a dentine-organ. These two structures are said to be derived from two distinct sources, the former from the oral epithelium and consequently is said to be ectodermal in origin, whereas, the latter is formed from the underlying sub-mucous tissue and is, therefore, mesodermal in derivation. At the point where a tooth is about to be developed an invagination or proliferation of the epithelial cells into the underlying mesodermal tissue is always to be seen. As this cone of epithelial cells proceeds inward, a papilla arises from the mesoderm beneath which grows into it thus causing it to assume a bell-shaped form with the concavity directed downward. The peripheral cells of the mesodermal papilla are said to form the dentine of the tooth for which reason this portion of the tooth germ receives the name of dentine-organ. The enamel-organ is that portion of the tooth germ which is formed from the epithelium. It has been well established by investigators that the enamel-organ is derived from the lowermost layer of the epithelium and that the enamel, when present, is formed from the epithelial cells which immediately invest the dentine papilla.

Investigators have shown that although there are many differences of detail arising from the various situations in which teeth develop in fishes there is, nevertheless, a great uniformity which pervades all that have been examined, and, that the method of development agrees essentially with that of other vertebrates. O. Hertwig ('74), who established the homology

of the teeth of vertebrates with placoid scales of elasmobranchs, claimed that in sharks the placoid teeth are successional and that each tooth germ, so far as the enamel-organ is concerned, develops from a general tooth-band. In this respect, the development of placoid teeth harmonizes with that of the mammalian tooth. Tomes ('23, p. 149) pointed out that whereas in the elasmobranchs each tooth germ, so far as the enamel organ is concerned, is derived from the general tooth-band, in teleost fishes "each enamel-germ apparently often arises independently, and, as it were, de novo." Miss Carlsson ('94), on the other hand, stated that in teleosts an enamel-ridge extends uninterruptedly along the whole length of the tooth-bearing bones and that the teeth do not develop in a continuous series, but new ones appear from the unexhausted enamel-ridge between those already formed. According to Miss Degener ('24) the teeth of Amia calva develop in essentially the same manner as placoid scales, though they are developed throughout all layers of the mucous membrane. She considers these placoid scale-like structures as representing intermediate steps between the lowest and highest forms of fishes. Röse ('94) distinguished three stages of tooth development in fishes: the first he called the free papilla or placoid stage, in which the primordia of the teeth develop in the mucous membrane in the same manner as placoid scales; the second he designated as the cone stage, in which the mucous membrane sends downward a separate tooth germ for each tooth primordium; the third or permanent stage is characterized by a dental ridge from which the teeth develop in the same way as those in higher vertebrates.

Although a considerable amount of investigation has already been carried out on the development of the pharyngeal teeth of the carp, a review of the literature failed to reveal any reference to the origin of these structures from the germ-layer viewpoint. It is quite significant that investigators, notably Heincke ('73), Friedman ('97) and Stoss ('21), are in agreement as to the origin of the enamel-organs from the lowermost cylindrical cells of the pharyngeal mucous membrane. These authors agree that the tooth germ forms by a proliferation of the lowermost layer of the pharyngeal epithelium which dips into the underlying sub-mucous tissue, and, that the latter responds by growing papilla-like into the under thickened end of the proliferated cone until it is surrounded like a cap by the epithelium.

In the present investigation the first evidence of an enamel-organ was observed in a larva 4 hours after hatching (series 1). Figure 23 is a photograph of a sagittal section through the pharynx showing the appearance of the enamel-organ as well as the mucous membrane lining this region. The latter can be seen to be composed of two distinct layers, an inner layer of irregularly arranged flattened cells and an outer or deeper layer of regularly arranged columnar cells. The primordium of the enamel-organ is recognizable as a crescent-shaped arrangement of cells. The boundaries of these cells have been outlined in ink for clearness of delineation. This portion of the tooth germ appears to be typical in that it is forming by an invagination from the pharyngeal epithelium. As regards the type and source of the cells which enter into the formation of the enamel-organ, the following significant facts are observed: the cells, which are arranged in a more or less orderly definite formation, are columnar in shape; these cells are not only similar to but are connected with the columnar cells which make up the lowermost stratum of the pharyngeal mucous membrane; the inner flattened layer of cells of the pharyngeal mucous membrane does not take part in the process of invagination but passes uninterruptedly over this point.

An examination of subsequent stages fails, likewise, to reveal any evidence of the inner flattened layer participating in the further development of the enamel-organ. In figure 24, which is a photograph of a sagittal section through the pharynx of a larva 18 hours after hatching, the tooth germ has advanced considerably in development. The dentine-organ has already begun to form as is evidenced by the rather large mesodermal papilla. The latter is invested by the enamel-organ which is recognizable as a bell-shaped structure made up of large columnar cells. The inner flattened layer of the pharyngeal mucous membrane can be readily made out in this figure. It appears as a continuous layer lining the pharyngeal cavity and does not seem to take part in the formation of the enamel-organ. By 25 hours after hatching (Fig. 25) the mesodermal papilla has increased in length considerably and has already begun to secrete a homogeneous matrix, the dentine of the future tooth. The enamel-organ has developed proportionately so that it has become folded upon itself thus converting it into a two-layered investing membrane. The columnar shape of the cells in the enamel-organ is very apparent in this figure and there

is no evidence of any flattened cells excepting those lining the lumen of the pharyngeal cavity. The continuity of the columnar cells, which make up the enamel-organ, with those in the deeper layer of the pharyngeal mucous membrane is shown at the point E. o... The columnar cells of the enamel-organ which immediately invest the dentine papilla have begun to assume a cylindrical form. The white area situated between these cylindrical cells and the tip of the dentine papilla suggests the presence of enamel. However, a careful examination under oil immersion failed to establish this point.

According to Tomes ('23, p. 149) the after-history of the enamel-organ depends much on the character of the tooth which is to be formed. If no enamel, or but a rudimentary coat of enamel, is to be formed, he claims, that the cells of the enamel-organ remain small and insignificant, whereas, if a partial investment of enamel is found upon the perfected tooth the cells of the enamel-organ attain a very considerable size opposite to the apex of the dentine-papilla, where the enamel cap is to be, and, that below this the investing cap of the enamel-organ becomes rudimentary. In figure 26, which is a photograph of a developing tooth 44 hours after hatching, the latter condition is suggested. The cells of the enamel-organ which immediately invest the apex of the dentine-papilla have attained a considerable size, whereas, below this point they have become greatly reduced. The dentine is now well marked out. Its apex appears to be capped with an enamel tip, however, this could not be absolutely determined. In the last analysis the question as to whether or not enamel is formed on these teeth is of no great importance in determining the main points under consideration, since, as Tomes ('23, p. 134) asserts, the presence of an enamel-organ is of universal occurence and is independent of any subsequent formation of enamel.

Discussion.

The problem of the specificity of the germ layers, especially as regards the potency of the endoderm and its capacity to form structures which are generally conceived to be derived from ectoderm, has long been a subject for investigation. As already indicated, investigators have attacked this problem from various angles. The adherents of the doctrine of the specificity of the germ layers, who hold that the presence of teeth in any

region is an accurate criterion for the existence of ectoderm in that region at some time in development, have employed the fact of the existence of teeth in the pharyngeal cavity of certain fishes, a region considered to be endodermal in origin, as evidence to support the hypothesis that ectoderm migrates into this region. Some merely assume that the ectoderm migrates posteriorly after the rupture of the oral plate, others that it arises from the invaginated ectoderm during the formation of the gill-slits. On the other hand, there are those who think that these so-called ectodermal derivatives arise in situ from the endoderm and deny any evidence of ectodermal migration either by way of the oral or pharyngeal clefts, at least not before the primordia of these structures are formed.

The problem of the germ-layer origin of the pharyngeal teeth of the carp, therefore, hinges on the mode of formation of the foregut as well as the derivation of its mucous membrane lining. The study of the development of the foregut shows that it is derived from endoderm which is completely established as a connected unicellular layer by 23 hours after fertilization. The results of the study of the method of development of the mouth and gill-slits present evidence that flattened cells from the epidermal stratum migrate inward to the oro-pharyngeal cavity and furnish its lining. The mucous membrane lining this cavity is, therefore, made up of two types of cells, each derived from distinct sources, an inner layer of flattened cells, which is derived from the epidermal stratum, and an outer or deeper layer of columnar cells, which represents the original endodermal layer.

The study of the development of the pharyngeal teeth shows that these teeth develop according to the typical vertebrate method, that is by an invagination of the lowermost epithelial layer of the pharyngeal mucous membrane forming an inverted cup-shaped enamel-organ which invests a mesodermal papilla, the dentine-organ. Thus it seems quite conclusive that the enamel-organs of the pharyngeal teeth of the carp are derived from the pharyngeal endoderm, since the lowermost epithelial layer of the pharyngeal mucous membrane has been shown to be the original endodermal layer of the primordial foregut.

The results of this study not only present evidence that the deeper columnar endodermal cells of the pharyngeal mucous membrane are the real formative elements in the formation of

the enamel-organs, but, also show that the inner flattened ectodermal cells do not appear to take part in the process. Suppose, however, that in the absence of any evidence to the contrary, the flattened ectodermal epithelium does invaginate along with the endodermal epithelium to form the enamel-organ. In such an event the ectoderm still could not be considered as contributing to the formation of the teeth, since it has been well established by investigators that the lowermost layer of the epithelium forms the enamel-organs, and, that the enamel, when present, is formed from the inner cylindrical cells of this organ.

The question may be suggested as to the probable influence of the ectoderm on the endoderm in initiating the formation of the enamel-organs. If the ectodermal cells in the pharynx influence the endodermal cells in this process, how can we account for the presence of tooth-like structures in the œsophagus of certain fishes and snakes, since they lie beyond the limits of the invaginated ectoderm? The conclusions arrived at in this paper are that endoderm possesses the capacity per se to form the enamel-organs of the pharyngeal teeth.

The question also arises as to whether the pharyngeal teeth of the carp are homologous with the typical vertebrate teeth. Obviously the answer depends upon the exact meaning of the term homology. If homologous structures are to be considered as those which have the same germ-layer origin then the answer is in the negative, since it has been shown in the present paper that the enamel-organs of the pharyngeal teeth of the carp are derived from endoderm, whereas, in the higher vertebrates they are formed from ectoderm. As the opponents of the doctrine of the specificity of the germ layers have repeatedly asserted, similarity does not necessarily prove that structures originate from the same germ layer. On the other hand, the pharyngeal teeth may be considered similar to the teeth of higher vertebrates, since they answer to the description and definition of a tooth as defined by Waldeyer ('72, p. 321) "The anatomical model of a vertebrate animal is a large papilla of the mouth or of the pharyngeal mucous membrane, which, in consequence of chemical and histological conversion of its constituents, has acquired a remarkable degree of hardness."

SUMMARY.

1. The epidermal stratum becomes differentiated by 10 hours after fertilization.

2. The primitive hypoblast arises 13 hours after fertilization by a proliferation from the mass of cells at the posterior middle point of the blastoderm.

3. By 19 hours after fertilization the anterior margin of the primitive hypoblast has extended anteriorly until it comes into contact with the ectoderm ventral and anterior to the future head-region.

4. Differentiation of the endoderm occurs by a process of flattening and later separation from the overlying primitive hypoblast. The remaining portions of the latter give rise to the notochord in the middle line and to the mesoderm on each side of this.

5. The endoderm is completely established as a connected unicellular layer by 23 hours after fertilization. Its anterior extremity comes into contact with the ectoderm anterior and ventral to the future fore-brain area. At this stage the endodermal cells in the region of the pharynx have assumed a columnar shape, whereas, in the extreme anterior portion of the primordium of the foregut they are flattened.

6. By 30 hours after fertilization the single endodermal layer, which can be recognized by means of the columnar shape of its cells, begins to rise up on either side to form lateral, obliquely directed folds in the region of the pharynx. These folds represent the pharyngeal folds which later contribute to the formation of the gill-slits.

7. The further course of development of these folds shows that their apices extend laterally and dorsally until they come into contact with the nervous layer of the ectoderm. The latter responds by sending inward a wedge-shaped proliferation into the apices of the folds. This results in the formation of continuous folds, made up laterally of ectoderm and medially of endoderm, the boundary between the two germ layers being indistinct.

8. Subsequent stages show that cleft-like lumina appear in these folds, beginning laterally and extending medially. In the meantime the epidermal stratum migrates inward by way of these lumina to the solid depressed foregut. The pharyngeal

clefts thus come to be lined with an inner layer of flattened cells from the epidermal stratum and a deeper layer of columnar cells, the original endodermal layer.

9. Simultaneous with the formation of the gill-slits the foregut closes ventrally. This is brought about by the base of each pharyngeal fold growing medially and fusing with its fellow of the opposite side. The dorsal and ventral rows of cells thus formed are at first firmly pressed against each other without any evidence of a lumen between them.

10. Later stages show that scattered lumina appear between these two layers of cells and that these unite eventually thus establishing the lumen of the foregut.

11. With the appearance of a lumen in the foregut, the endoderm assumes the same structure as in the lumina of the pharyngeal clefts, that is, its lining then consists of an inner layer of flattened cells and an outer or deeper layer of columnar cells.

12. Observations on the development of the mouth present evidence to support the views of Dohrn and Ryder that the mouth develops similarly to the gill-slits. The mouth breaks through in the angle between the head and the anterior ectodermal wall of the yolk sac without any evidence of a stomodaeum. Furthermore, oral folds, which are similar in character to pharyngeal folds, extend diagonally forward and come into contact with the inner layer of the ectoderm posterior and ventral to the primordia of the eyes. Clefts form here similar to pharyngeal clefts and the epidermal stratum appears to migrate inward by way of these clefts in the same manner as in the pharyngeal clefts. The so-called oral clefts as well as the pharyngeal clefts appear to form by invaginations of the epidermal stratum thus establishing potential openings. In both instances the epidermal stratum migrates inward to the oro-pharyngeal cavity thus contributing to its inner lining. It is evident, therefore, that the inner lining of the oro-pharyngeal cavity is derived from ectoderm and that the deeper epithelial layer of the mucous membrane of this region represents the original endodermal layer.

13. Observations on the development of the pharyngeal teeth reveal that the enamel-organs are derived from the deeper coloumnar layer of the pharyngeal epithelium and are, therefore, endodermal in origin, since the deeper columnar

layer of the mucous membrane has been demonstrated to represent the original endodermal layer of the primordial foregut. Furthermore no evidence could be found of the inner flattened layer of cells contributing to the formation of the enamel-organs. The conclusion arrived at is that the enamel-organs of the pharyngeal teeth of the carp are, therefore, endodermal in origin.

BIBLIOGRAPHY.

ADAMS, A. E. 1924. An experimental study of the development of the mouth in the amphibian embryo. Jour. Exp. Zool., Vol. 40.

BALFOUR, F. M. 1878. A monograph on the development of elasmobranch fishes. MacMillan and Co., London.

1881. A treatise on comparative embryology. MacMillan and Co., London.

BAUME, A. 1882. Odontologische Forshungen. Leipzig.

BOAS, J. E. 1879. Die Zähne der Scaroiden. Zeitsch. f. Wiss. Zool., Vol. 32.

CARLSSON, A. 1894. Uber die Zahenentwickelung bei einige Knochenfischen. Zool. Jahr., Vol. 8.

COOK, N. AND NEAL, H. V. 1921. Are the taste buds of elasmobranchs endodermal in origin? Jour. Comp. Neur., Vol. 33.

DEGENER, L. M. 1924. The development of the dentary bone and teeth of Amia calva. Jour. Morph., Vol. 39.

DOHRN, A. 1882. Studien zur Urgeschichte des Wirbelthierkorpers. Mittheil. aus der Zool. Station zu Neapel., Vol. 3.

FAHRENHOLZ, C. 1915. Uber die Verbreitung von Zahnbildungen und Sinnesorganen im Vorderdarm der Selachier und ihre Phylogenetische Beurteilung. Jena. Zeits. f. Naturwiss., Vol. 53.

FRIEDMAN, E. 1897. Beitrag zur Zahnentwicklung der Knochenfische. Morph. Jahr., Vol. 4.

GEGENBAUR, C. 1898. Vergleichende Anatomie der Wirbelthiere. Leipzig. Vol. 2.

GOETTE, A. 1875. Die Entwickelungsgeschichte der Unke. Leipzig. 1901. Uber die Kiemen der Fische. Zeits. f. Wiss. Zool., Vol. 69.

GOLOVINE, E. 1902. Sur le fixage du neutralroth. Zeits. f. Wiss. Mikros., Vol. 19.

GREIL, A. 1906. Uber d. Homologie der Anamnierkiemen. Anat. Anz., Vol. 28.

HEINCKE, F. 1873. Untersuchungen über die Zahnen niederer Wirbelthiere. Zeits. f. Wiss. Zool., Vol. 23.

HERTWIG, O. 1874. Ueber Bau und Entwickelung der Placoidschuppen und der Zahne der Selachier. Jena. Zeits. f. Natur., Vol. 8.

IMMS, A. D. 1904. Notes on the gill-rakers of the Spoonbill Sturgeon, Polyodon spathula. Proc. Zool. Soc. London, Vol. 2.

1905. On the oral and pharyngeal denticles of elasmobranch fishes., Ibid., Vol. 42.

JACOBSHAGEN, E. 1911. Untersuchungen uber das Darmsystem der Fische. Jena. Zeits. f. Natur., Vol. 40.

1912. Ibid., Vol. 42.

JENKINSON, J. W. 1906. Remarks on the germinal layers of vertebrates. Memoirs and Proc. Manchester Literary and Philosophical Soc., Vol. 50.

KERR, J. G. 1902. The development of Lepidosiren paradoxa. Quart. Jour. Micro. Soc., Vol. 46.

KINGSLEY, J. S. 1926. Outlines of comparative anatomy of vertebrates. P. Blakiston's Son & Co., Philadelphia.

LANKESTER, E. RAY. 1909. A treatise on Zoology. Part IX. A. & C. Black, London.

MOROFF, T. 1901. Ueber die Entwicklung der Kiemen bei Knochenfische. Arch. f. Mikro. Anat., Vol. 60.
1904. Uber die Entwicklung der Kiemen bei Fischen., Ibid., Vol. 64.
NUNN, E. 1882. On the development of the enamel of the teeth of vertebrates. Proc. Royal Soc. London, Vol. 34.
OELLACHER, J. 1872. Beitrage zur Entwicklungsgeschichte der Knochenfische, nach Beobachtungen am Bach forellenei. Zeits. f. Wiss. Zool., Vol. 22.
1873. Ibid., Vol. 23.
OPPEL, A. 1897. Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbelthiere. Band II. Schlund und Darm. Jena.
PICTET, A. 1909. Contribution histologique du tube digestif des Poisons Cyprinoides. Rev. Suisse de Zool., Vol. 17.
PRINCE, E. E. 1892. The development of the teeth in the Labridæ. Meeting British Assoc. Edinburgh, 62nd. Meeting.
RIDEWOOD, W. G. 1896. The teeth of fishes. Nat. Sci., Vol. 8.
RÖSE, C. 1894. Ueber die Zahnentwickelung der Fische. Anat. Anz., Vol. 9.
RYDER, J. A. 1882. A contribution to the embryology of osseous fishes. Report of U. S. Fish Comm., Washington, 1884.
STEINHARD, O. 1903. Placoidschuppen in Mund und Rachenhohle der Plagiostomen. Arch f. Natur., Vol. 69.
STOSS, H. 1903. Die Schlundzahne des Karpfens. Zool. Jahrb., Vol. 42.
THOMPSON, A. H. Comparative dental anatomy.
TOMES, C. S. 1923. A manual of dental anatomy. J. & A. Churchill, London. 8th. edition.
TURNER, W. 1880. The structure of the comb-like branchial appendages and of the teeth of the basking shark. Jour. of Anat. and Physiol., Vol. 14.
WALDYER, W. 1872. Structure and development of the teeth. Strickler's Manual of Histology. Wm. Wood & Co., N. Y.
WIEDERSHEIM, R. 1886. Lehrbuch der Vergleichende Anatomie der Wirbelthiere. Jena.
WILSON, H. V. 1889. Embryology of the sea-bass (Serranus atrarius). Bull. of the U. S. Fish Comm., Vol. IX.
WORTMAN, J. L. 1886. Comparative anatomy of the teeth of the vertebrates. American System of Dental Surgery, Vol. I.

EXPLANATION OF PLATES.

PLATES I AND II.

These Figures were made with the aid of a Spencer drawing apparatus. Magnification × 300.

Figs 1 to 4. Sagittal sections through the blastoderm 10, 12, 13 and 19 hours, respectively, after fertilization.

Fig. 5. Transverse section through the blastoderm 21 hours after fertilization.

Fig. 6. Midsagittal section through an embryo 23 hours after fertilization.

Figs. 7 to 12. Transverse sections through the pharynx 30, 32, 35, 36, 39 and 56 hours, respectively, after fertilization.

Fig. 13. Sagittal section cut far laterally through the anterior region of an embryo 57 hours after fertilization.

Fig. 14. Transverse section through the pharynx of an embryo 78 hours after fertilization.

Fig. 15. Transverse section through the oral end of the foregut 27 hours after fertilization.

Fig. 16. Sagittal section cut far laterally through the anterior region of an embryo 59 hours after fertilization.

PLATE III.

Fig. 17. Sagittal section cut far laterally through the pharyngeal region of the same embryo as in Fig. 13. × 900.

Fig. 18. Sagittal section through the anterior region of an embryo 80 hours after fertilization. × 100.

Fig. 19. Mid-sagittal section through an embryo 79 hours after fertilization. × 100.

Fig. 20. Sagittal section cut far laterally through the same embryo as in Fig. 19. × 100.

Fig. 21. Transverse section through the anterior end of the same embryo as in Figure 14. × 100.

Fig. 22. Photograph of the inferior pharyngeal bones of the last branchial arch, showing the pharyngeal teeth of an adult carp. × 2.

PLATE IV.

Fig. 23. Sagittal section through the pharyngeal region of a larva 4 hours after hatching, showing the formation of an enamel-organ. × 900.

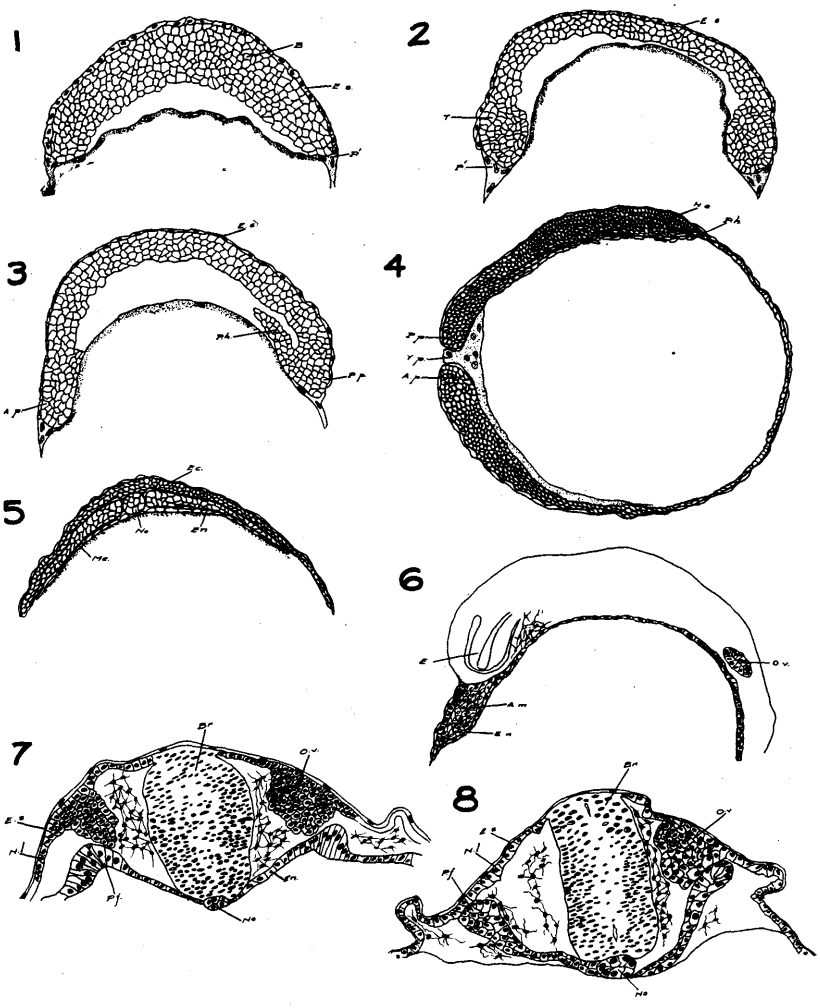
Fig. 24. Sagittal section through the pharyngeal region of a larva 18 hours after hatching, showing the further development of the tooth-germ. × 900.

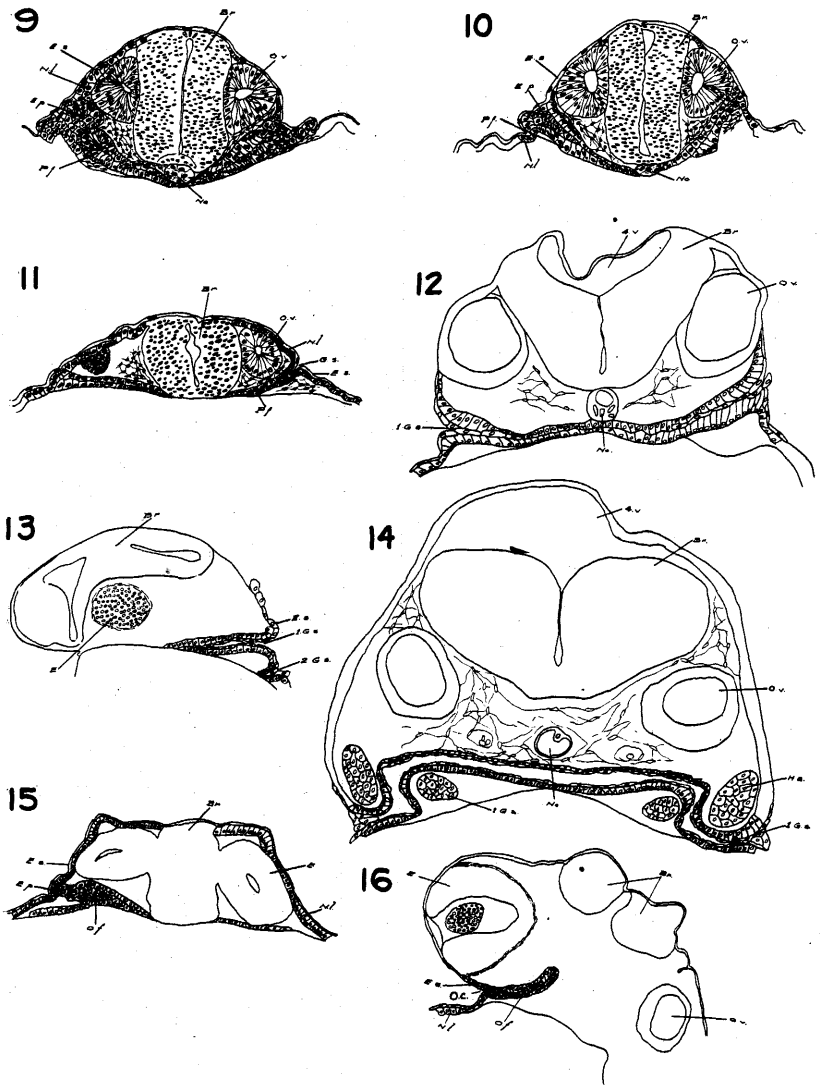
Fig. 25. Section through the tooth-germ of a larva 25 hours after hatching. × 900.

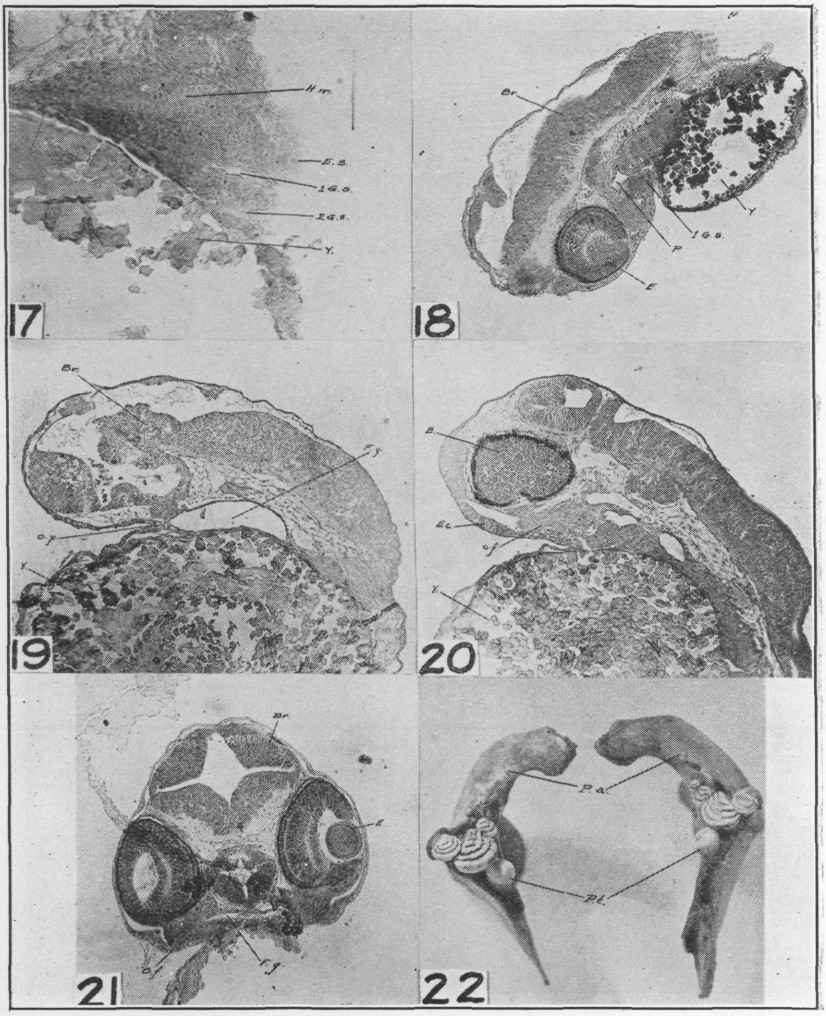
Fig. 26. Section through a tooth-germ of a larva 44 hours after hatching. The small tip, E. t., appears to be a separate enamel tip. However, this is not certain, hence the interrogation point. × 1220.

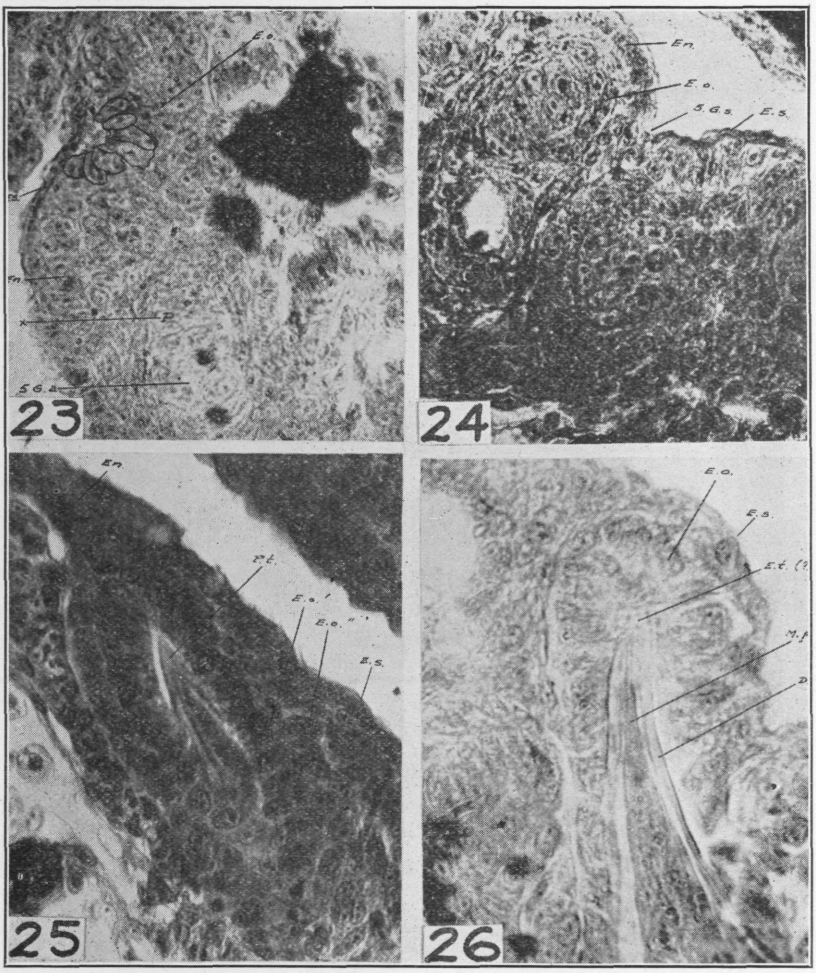
ABBREVIATIONS.

A. m.—anterior mass of mesoderm.
A. p.—anterior pole of blastoderm.
B.—blastoderm.
Br.—brain.
D.—Dentine.
E.—eye.
Ec.—ectoderm.
En.—endoderm.
E. o.—enamel-organ.
E. o.'—inner layer of enamel-organ.
E. o.''—outer layer of enamel-organ.
E. p.—proliferating ectodermal plug.
E. s.—epidermal stratum.
E. t.—enamel tip (?).
F. g.—foregut.
G. a.—gill-arch.
G. s.—gill-slit.
H. a.—hyoid arch.
H. e.—head of future embryo.
H. m.—hyomandibular arch.
Me.—mesoderm.
M. p.—mesodermal papilla (dentine-organ).
N. l.—nervous or inner layer of ectoderm.
No.—notochord.
O. c.—oral-cleft.
O. f.—oral fold.
O. p.—oral plate.
O. v.—otic vesicle.
P.'—periblast.
P.—pharynx.
P. a.—pharyngeal arch.
P f.—pharyngeal fold.
P. h.—primitive hypoblast.
P. p.—posterior pole of blastoderm.
P. t.—pharyngeal tooth.
4V.—4th ventricle.
Y.—yolk.
Y. p.—yolk plug.


1
2
3
4
5
6
7
8


9
10
11
12
13
14
15
16


17
H m.
E. s.
1 G. s.
2 G. s.
Y.
18
Br.
Y
1 G. s.
P
E.
19
Br.
E
Ec
o f
Y.
20
E.
Ec.
o f
Y.
21
Br.
E
o f
f. j.
22
P. a.
Pl.


E.o.
En.
P
S.G.s.
23
En.
E.o.
S.G.s.
E.s.
24
En.
Et.
E.o.'
E.o.''
E.s.
25
E.o.
E.s.
E.t. (?
M.f.
D
26